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# Application of Novel Microorganism-Based Formulations as Alternative to the Use of Iron Chelates in Strawberry Cultivation

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**Abstract:** The strawberry is a low-growing, herbaceous perennial plant, sensitive to iron deficiency. The iron deficiency represents a nutritional disorder, leading to a decreased content of photosynthetic pigments, which determines the yellow color characteristic of chlorotic leaves. Therefore, in calcareous soils, the use of synthetic iron chelate is often mandatory in strawberry cultivation. The employment of novel microorganism-based formulations as alternatives to the use of iron chelates, was evaluated during strawberry cultivation by monitoring the morpho-biometric parameters, chlorophylls, the iron content in leaves and roots, and the Fe chelate reductase activity involved in absorption of iron during the chlorosis event in plants using the strategy I. The experimental design envisaged growing strawberry seedlings on an inert substrate (pumice), irrigated with Hoagland solution iron-free, with a 12 h photoperiod. After 42 days, at the first appearance of chlorosis symptoms, plants were transplanted into a calcareous soil, and after seven days, they were treated, by a single application, with a microorganism-based formulations (MBF), an inoculum (In) of *Trichoderma* spp. and *Streptomyces* spp., or Sequestrene (Sq). Strawberry plants were sampled and analyzed at 5, 10, 15, and 20 days from the treatments. The results showed that microorganism-based formulations positively affected the strawberry seedlings, by reducing the chlorosis symptoms, producing comparable effects to the Sequestrene treatment.

**Keywords:** EDDHA; FC-R; Fe-deficiency; *Fragaria* × *ananassa*; *Glomus*; *Trichoderma*; *Streptomyces*



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## 1. Introduction

Iron deficiency is an important nutritional disorder in plants, resulting from the altered acquisition and use of Fe, rather than from a low level of Fe in soils, determining as the primary effect a decreased content of photosynthetic pigments, which leads to the characteristic yellow color of chlorotic leaves [1]. The ferric chlorosis is determined by iron necessity for the correct functionality of proteins involved in the synthesis of chlorophylls. Indeed, the synthesis of δ-aminolevulinic acid, precursor of chlorophylls, is regulated by the presence of iron [2]. Iron is also necessary for the synthesis of the protochlorophyllide from Mg-protoporphyrin. Moreover, in the thylakoid membrane, 20 atoms of iron are needed for photosynthetic electron transport chain of the PSII and PSI photosystems [3–5]. Such metabolic disorders induced by Fe deficiency cause chloroplast disorganization. This effect is shown by decreasing of photosynthetic units, granules, and stromal lamellae of the chloroplast, and by the decrease of thylakoids [6].

Different soil factors, including alkaline pH (nearly 8.0), free $CaCO_3$, and $HCO_3^-$, influence the iron availability for the plants [7]. In fact, in calcareous soils, where free $CaCO_3$ reacts with soil moisture and $CO_2$ leads to the production of $HCO_3^-$, Fe deficiency represents a crucial factor for crop growing [8,9]. Therefore, iron deficiency is one of the main factors related to the reduction of crop yield in calcareous soils, making the Fe uptake by the plants difficult due to its physical and chemical properties [10,11]. In this context, the microorganisms of the soil have an important role, as mineralizing the organic matter determines the release of iron cations $Fe^{2+}$ and $Fe^{3+}$. In the soil, the redox equilibrium is shifted from the reduced form (ferrous iron) ($Fe^{2+}$) to the oxidized form (ferric iron) ($Fe^{3+}$), determining the precipitation of cations as ferric hydrates. Iron is absorbed by plants in cationic form, and $Fe^{2+}$ represents the favorite form [12]. The iron bioavailability in the soil is correlated to a balancing between the ions and free oxides as a consequence of pH and redox potential [13]. Therefore, the iron availability in the soil and rhizosphere depends on its concentration in the soil solution, and on the ability of the solid phase to supply the liquid phase of soluble forms through the balance between processes of solubilization/precipitation and dissociation/formation of the complex, occurring in the soil [13].

The plants have acquired specific absorption systems for iron to cope with its deficiency. The dicotyledonous and monocotyledon not graminaceous plants use a system strategy named "strategy I", in which the plants secrete in the soil specific organic compounds, such as malic acid and citric acid, together with protons (due to membrane ATPase pumps) [12]. These substances decrease the soil pH and form more stable chelate of the previous iron chelate, allowing them to reduce iron to the $Fe^{2+}$ form at the cell membrane level. The ability to reduce the Fe(III) to Fe(II) is attributable to the membrane enzyme named Fe chelate reductase, which acts as a NADH-dependent reductase. This result is stimulated by the decrease of pH induced by the activity of protonic pumps [14].

"Strategy II" is active in to graminaceous plants, which secrete chelating organic substances in the soil called phytosiderophores (mugineic acid, avenic acid, etc.). The phytosiderophores have the ability to chelate $Fe^{3+}$ and carry it inside the cell through the membranes [15].

It has also been shown that humic substances (HS) have an important role in Fe assimilation. In fact, soluble Fe-HS complexes, naturally present in the soil, can promote iron acquisition by providing a readily available iron in the rhizosphere and by directly affecting plant physiology through mechanisms involved in Fe acquisition acting at the transcriptional and post-transcriptional level in the plant [16,17].

Moreover, soil microorganisms, such as a mixture of *Trichoderma* spp. and *Streptomyces* spp., are also able to produce siderophores, which are similar to those produced by the plants [18,19]. Siderophores are small and high-affinity iron-chelating compounds that microorganisms synthesize under iron-deficiency stress in order to guarantee the growth and development of their cells by increasing soluble iron in the soil and making it available for themselves [20]. These types of secondary metabolites promote the chelation of iron, making it more available for the microorganisms the produced it, and at same time for the plants [21,22]. Indeed, Zhao et al. [18] found that in *Arabidopsis,* the siderophores produced by *Trichoderma asperellum* Q1 in the soil act by enhancing the conversion of poorly soluble Fe. In soil inoculated with *Trichoderma asperellum*, the soluble Fe and siderophores increased and the growth in cucumber seedlings grown in the inoculated soil were enhanced by increasing the ferric chelate reductase (FCR) activity in roots [23]. Therefore, the siderophores produced by several rhizosphere microorganisms may be very useful for promoting the absorption of iron by plants in an iron-deficient environment [24,25].

Iron deficiency represents a limiting growing factor for those horticultural crops, such as strawberry, requiring highly specialized knowledge and high external inputs [26,27]. Among the chelating agents, the most used for iron complexation is ethylenediamine-N (o, p-hydroxyphenylacetic) acid (EDDHA), and in particular its isomeric "orto" form, which guarantees the highest stability level of the ion. Other iron chelating agents are ethylene-

diaminetetraacetic acid (EDTA), ethylene diamine di(hydroxy methyl phenyl) acetic acid (EDDHMA), and ethylenediamine di (2-hydroxy-4 carboxyphenyl acetic) acid (EDDCHA). In particular, soil management, irrigation, and fertilizations are all crucial events in order to obtain favorable strawberry productions, although an excess of these compounds often results in environmental problems [27,28]. In addition, chemical compounds may also negatively affect the beneficial soil microorganisms, therefore horticulture is addressing the reduction of the use of these chemical compounds [29,30]. In this respect, FAO [31] suggested that in order to create a safe environment, agriculture must contribute to improving the living standards of all, especially the poorest, in an economically, socially, and environmentally sustainable manner.

The aim of this study was to verify the efficiency of new microorganism-based formulations (MBF and In) against iron deficiency, in order to evaluate the iron absorption efficiency by plants as well as Fe solubility in a calcareous soil. Finally, the efficiency of these commercial formulations was compared to the well-known effect of the iron chelate Sequestrene.

## 2. Materials and Methods

### *2.1. Plant Material and Growth Conditions*

The trials were conducted using strawberry plants (*Fragaria* × *ananassa* Duch. cv. 'Portola'), with bared roots, at the stage of two true leaves, provided by a local farmer in Maletto (Catania, Sicily, Italy). The seedlings were washed with distilled water, and transplanted into containers (30 × 45 × 20 cm) filled with an inert support (pumice) as substrate, sprinkled with 1 L of nutrient solution of Hoagland iron-deficient solution (5 mM $Ca(NO_3)_2$, 5 mM $KNO_3$, 1.0 mM $KH_2PO_4$, 2.0 mM $MgSO_4$, 46.0 mM $H_3BO_3$, 0.8 mM $ZnSO_4$, 0.4 mM $CuSO_4$, 9.0 mM $MnCl_2$, and 0.02 mM $MoO_3$), in order to induce chlorosis symptoms in strawberry seedlings. In each container, 5 strawberry plants, for a total of 85 seedlings for each of the three repetitions, were transplanted.

The pH of the nutritive solution was 6.4 ± 0.2 and the electrical conductivity (EC) was 2.2 ± 0.2 dS $m^{-1}$. Hoagland solution was periodically added to keep a constant pH and electrical conductivity in the solution. The experimentation was performed in May, using laboratory conditions in a climatic chamber, with a photoperiod of 12 h of light at room temperature (23–27 °C). SPAD values (SPAD-502 Leaf Chlorophyll Meter, Minolta Camera Co., Ltd., Osaka, Japan) were recorded every 7 days, as described in Barone et al. [32], to monitor the iron deficiency levels. After 42 days, at the first sign of chlorosis symptoms on a new leaf (with total chlorophyll content < 150 µmol $m^{-2}$), seedlings were transplanted into a plastic pot (diameter 20 cm, height 20 cm) filled with soil and grown for 7 days. After the treatments, plants were grown for a further 20 days.

### *2.2. Soil Characterization*

The substrate used in the experimental trials was a calcareous soil clay-loam (sand: 32%; silt: 30%; clay: 38%). The values of pH, electrical conductivity (EC), organic matter (OM), organic carbon (OC), cation exchange capacity (CEC), total $CaCO_3$, active $CaCO_3$, total iron (in aqua regia), extractable iron (in oxalate), available iron (present in exchange complexes), and readily available iron (present in soil solution) were previously determined (Table 1). The chemical characterization was carried out according to Violante [33] and Barone et al. [34]. Soil was chosen for its chlorosing power index (CPI), calculated as the ratio between active calcareous (mg/kg) and the square of extractable iron (ppm). If the calculated index is greater than 100, soil will potentially determine chlorosis symptoms in the crops [35]. Before transplant, in order to improve some soil physical characteristics (such as porosity and structure), bovine manure (0.5 kg/$m^2$) was added [36].

**Table 1.** Characteristics of soil subjected to the experimental design.

| Soil Properties | Pre-Treatment |
|---|---|
| pH | 8.1 ± 0.2 |
| E.C (dS/m) | 0.179 ± 0.010 |
| CEC (meq/100 g) | 18 ± 1.1 |
| O.M. (%) | 2.0 * ± 0.8 |
| O.C. (%) | 1.14 * ± 0.6 |
| Active $CaCO_3$ (%) | 19.5 ± 1.2 |
| Total $CaCO_3$ (%) | 37.1 ± 3.2 |
| Total Fe (mg/kg) | 665 ± 21 |
| Extractable Fe (mg/kg) | 41.38 ± 5.3 |
| Available Fe (mg/kg) | 1.96 ± 0.2 |
| Readily available Fe (mg/kg) | 0.20 ± 0.02 |
| CPI | 113.9 ± 10 |

* O.M: 1.7% before amendment; O.C: 0.97% before amendment.

### 2.3. Treatments

Seven days after the transplant, the following soil treatments were performed: (I) CNT, control (no treatment); (II) Seq, treatment with Sequestrene at the recommended dose of 2 g/$m^2$; (III) MBF, treatment with microorganism-based formulations at the recommended dose of 2 mL/$m^2$; (IV) In, treatment with inoculum of MBF + molasses, in dose of 2 mL/$m^2$.

As synthetic chelating was used, the Sequestrene® (Syngenta NK 138Fe, Basel, Switzerland) was composed of 3% total nitrogen, 15% $K_2O$, 6% soluble Fe, and 5.5% chelating Fe with EDDHA (3% as [o,o]). MBF consisted of 5% soluble Fe, 2% chelating Fe with EDDHA ([o,o]), and an inoculum composed of: organic matter, 0.0001% *Glomus* spp., $10^3$ UFC/g rhizosphere bacteria, $10^8$ UFC/g of *Trichoderma*, and >$10^8$ UFC/g *Streptomyces* spp. and *Trichoderma* spores. Inoculum (In) consisted of organic matter, 0.0001% *Glomus* spp., $10^3$ UFC/g rhizosphere bacteria; $10^8$ UFC/g of *Trichoderma asperellum*, and $10^8$ UFC/g *Streptomyces avermitilis* and $10^8$ UFC/g *Trichoderma asperellum* spores.

Soil treatments were performed in only one liquid application at the soil level. MBF was reconstituted by dissolving the inoculum in the nutritive base (according to manufacturer's instructions, the inoculum was added at 2%), diluted in water (200 mL), and applied at the recommended dose (as previously described).

Inoculation of the soil with microorganism was performed by diluting the inoculum (provided by the manufacturer) in water (the inoculum was added at 2%, in 200 mL), and by applying it directly into the soil.

Plants were treated 7 days after transplanting in the soil. For each thesis and replica, 20 seedlings were treated and monitored for 20 days. Every 5 days, samples from 5 plants for each thesis and replica were harvested and analyzed; consequently, the determination were performed at T5, T10, T15, and T20. T0 consisted of 5 plants harvested and analyzed before the treatments. For each thesis, 3 replicas were performed. During the experimental period seedlings were irrigated with a drip irrigation system.

### 2.4. Determination of Fresh and Dry Weights, and Chlorophylls

The strawberry seedlings were collected and roots and leaves were separated and weighted. For dry weight determination, plant samples were separately dried at 105 °C, until a constant weight was reached [37].

The chlorophyll content was monitored using the SPAD values (SPAD-502 Leaf Chlorophyll Meter, Minolta Camera Co., Ltd., Osaka, Japan) in accordance with Pestana et al. [38]. The measures were taken 5 times on at least 3 leaves for each plant. The SPAD values were converted into quantitative total chlorophylls (mmoli·$m^{-2}$) using a calibration curve. The calibration curve was made by analyzing portions of leaves with different degrees of chlorosis, measuring both SPAD values and chlorophyll content by spectrophotometric method of extraction in acetone described by Puglisi et al. [39].

### 2.5. Iron Content in the Plants

The plant iron content was measured in both leaves and root [40]. The samples were washed with deionized water with 0.01 M HCl solution. The plants were dried until constant weight at 105 °C and subsequently incinerated in a muffle furnace at 500 °C for 24 h. The resulting ashes were dissolved in 10 mL of 1% (*v*/*v*) $HNO_3$, filtered with Whatman 0.45 µm filters (Whatman® Schleicher & Schuell, Dassel, Germany), and the Fe content was determined by atomic absorption (Perkin Elmer, Norwalk, CT, USA).

### 2.6. Ferric Chelate Reductase Activity in Roots

The roots (about 2 cm) were cut; washed with distillated water; immersed in a solution containing 0.5 mM $CaSO_4$, 0.25 mM Fe(III)-EDTA, 0.6 mM bathophenanthroline disulfonate (BPDS), and 10 mM Mes-KOH (pH 6); and kept in the dark for 60 min [40]. The activity was measured by monitoring the increase in absorbance of the solution in contact with the root at 535 nm, due to the root's capacity to reduce the Fe(III)-EDTA with the production of colored Fe(II) batophenantrolina complex (BPS-$Fe^{2+}$) [41]. After sample centrifugation, the absorbance was measured by spectrophotometer (Jasco, Tokyo, Japan). The nmoles of BPS-$Fe^{2+}$ produced were calculated using the molar extinction coefficient equal to 22.14 mM $cm^{-1}$ [42].

### 2.7. Statistical Analysis

The data of fresh and dry weights, iron content, chlorophyll content, and Fe chelate reductase activity were analyzed by one-way ANOVA (one way, $p < 0.05$), followed by a Tukey test.

## 3. Results

Fresh and dry weights of strawberry seedlings were monitored for 20 days and measured every five days (Figures 1 and 2).

As shown in Figure 1A, the leaf fresh weight after 5 days (T5) from the treatment significantly increased (about 88% than the control) only in strawberries that were treated with Sequestrene (Seq). After 10 days (T10) from the treatment, seedlings that were treated with MBF also began to significantly increase their leaf dry weight of about 58% with respect to the control (Figure 1A). Finally, from 15 days (T15) to the end of the experiment (T20), the MBF effect on the fresh weights of the leaves was comparable to the effect induced by Seq, showing an increase in the final fresh weight of around 62% compared to the control (Figure 1A). On the contrary, the inoculum (In) showed a significantly effect than the control only at the end of the experimental period (T20), although it was always significantly lower than treatments with Seq and MBF (Figure 1A).

With regards to the root fresh weights, Figure 1B shows an evident effect due to microorganism action of MBF (MBF and In). The plants treated with Seq showed root fresh weights statistically not significant with respect to the plants treated with MBF and In, except at the time T10, in which the root fresh weights of seedlings treated with Seq were lower than those measured in strawberries treated with BMF and In (Figure 1B).

The leaf dry weights (Figure 2A) showed that sequestrene seems to be the best treatment, showing at T20 a significant increase with respect to all the others (around 180%, 32%. and 101% for CNT, MBF, and In, respectively) (Figure 2A). As regards the root dry weights, the trend observed was very similar to that observed for fresh weights (Figure 2B).

Figure 3A,B shows the iron content (g $Kg^{-1}$ dry matter) in the leaves and root during the experimental trials. As expected, the highest values were registered for Seq and MBF treatments, both in leaves and in roots, with values similar for the two theses, except at the time T5 (Figure 3A,B), in which the Seq treatment drastically increased the iron level in both tissues. As a consequence of iron content increase in leaves (Figure 3), total chlorophyll contents (Figure 4) in seedlings treated with Seq and MBF have shown significantly higher values with respect to the control and In, recording a percentage increase of 177% and 180%, respectively. The only exception was found at the time T5, at which point chlorophylls in

the leaves treated with Seq were significantly higher than those measured in plants treated with MBF (Figure 4). The increase in the iron and chlorophyll contents was immediately evident at the visual analysis of the leaves of treated and untreated strawberry seedlings (Figure 5).

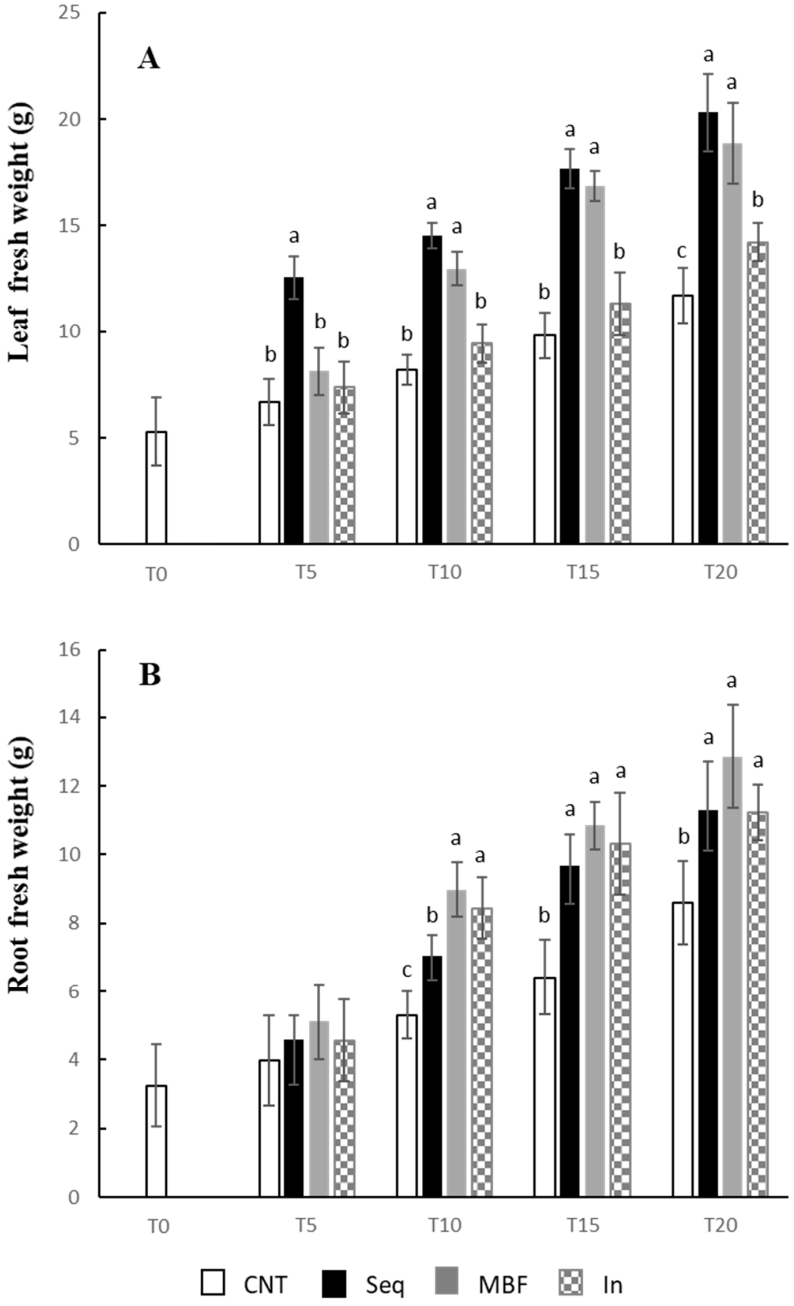


**Figure 1.** Fresh weights (g) of leaves (**A**) and roots (**B**) of strawberry plants. Error bars indicate standard deviation ($n$ = 5). Sampling was performed at T0 (before treatments), T5 (5 days after the treatment), T10 (10 days after the treatment), T15 (15 days after the treatment), T20 (20 days after the treatment). CNT: control, Seq: sequestrene, MBF: microorganism-based formulation, In: inoculum of MBF. Values within each sampling time followed by different letters are significantly different ($p < 0.05$).

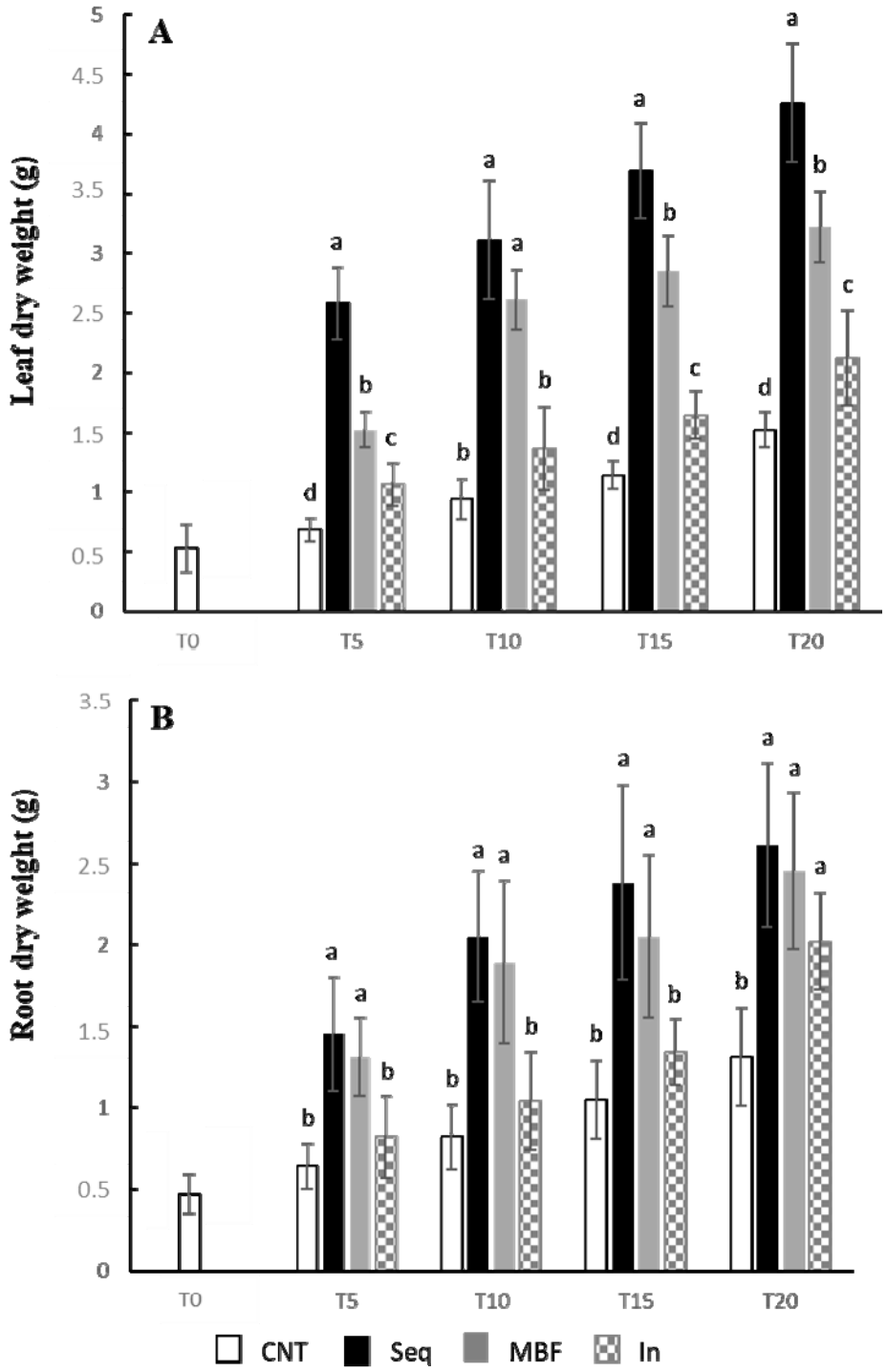


**Figure 2.** Dry weights (g) of leaves (**A**) and roots (**B**) of strawberry plants. Error bars indicate standard deviation ($n$ = 5). Sampling was performed at T0 (before treatments), T5 (5 days after the treatment), T10 (10 days after the treatment), T15 (15 days after the treatment), T20 (20 days after the treatment). CNT: control, Seq: sequestrene, MBF: microorganism-based formulation, In: inoculum of MBF. Values within each sampling time followed by different letters are significantly different ($p < 0.05$).

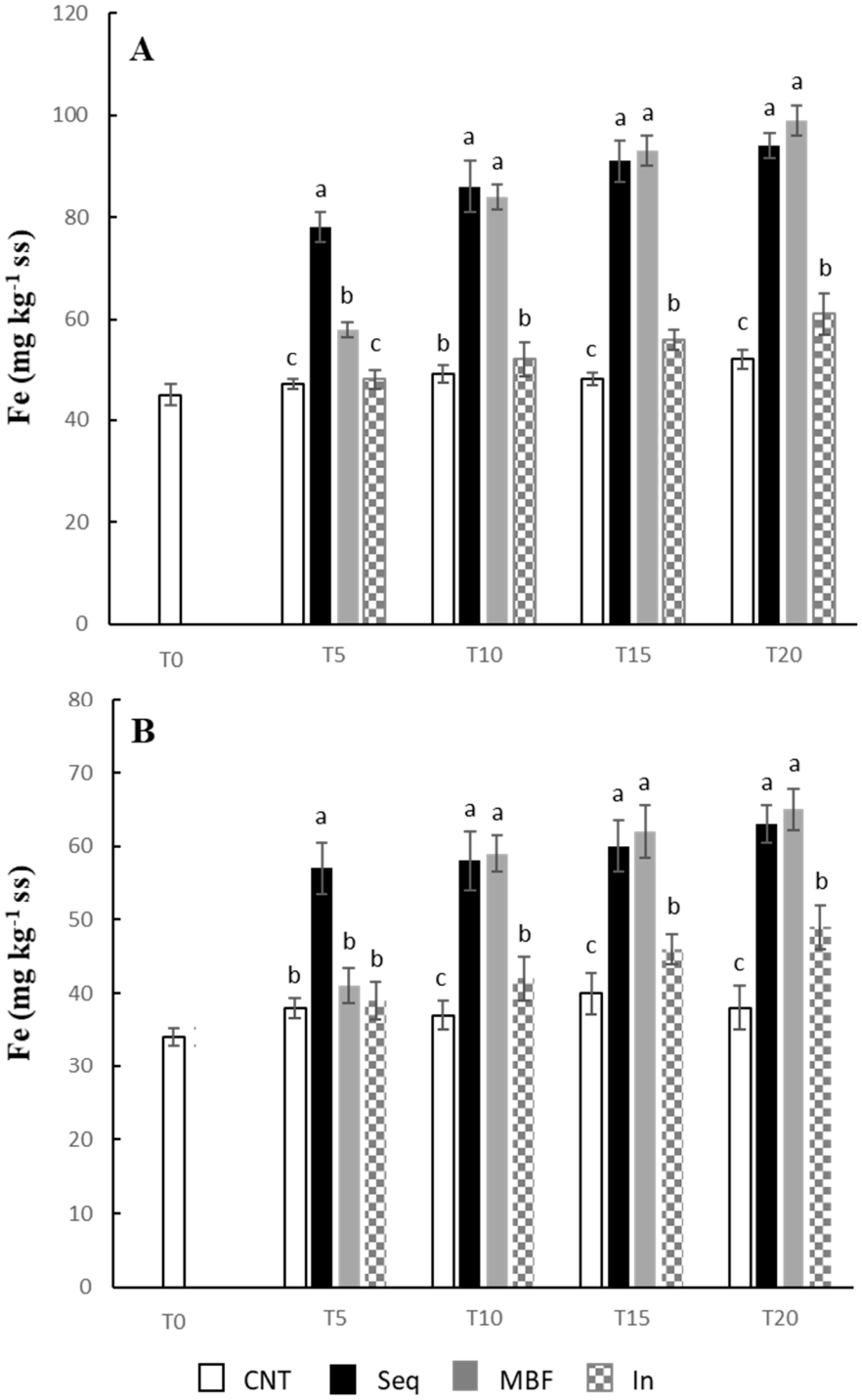


**Figure 3.** Iron content (mg $kg^{-1}$ ss) in leaves (**A**) and roots (**B**) of strawberry plants. Error bars indicate standard deviation ($n = 5$). Sampling was performed at T0 (before treatments), T5 (5 days after the treatment), T10 (10 days after the treatment), T15 (15 days after the treatment), T20 (20 days after the treatment). CNT: control, Seq: sequestrene, MBF: microorganism-based formulation, In: inoculum of MBF. Values within each sampling time followed by different letters are significantly different ($p < 0.05$).

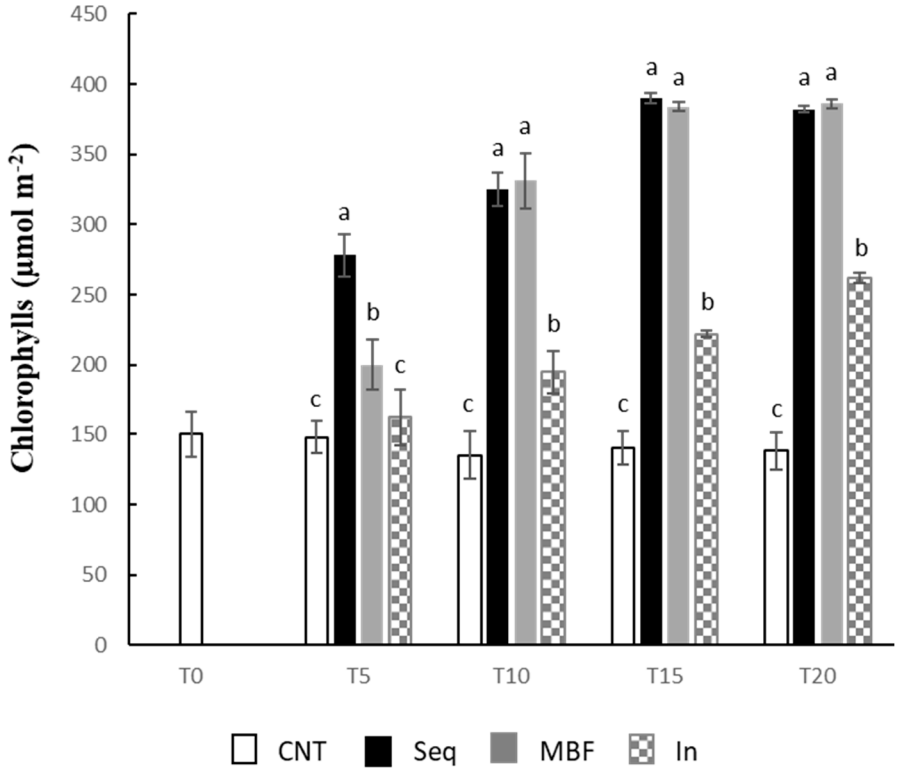


**Figure 4.** Chlorophyll content (µmoles $m^{-2}$) in leaves of strawberry plants. Error bars indicate standard deviation ($n$ = 5). Sampling was performed at T0 (before treatments), T5 (5 days after the treatment), T10 (10 days after the treatment), T15 (15 days after the treatment), T20 (20 days after the treatment). CNT: control, Seq: sequestrene, MBF: microorganism-based formulation, In: inoculum of MBF. Values within each sampling time followed by different letters are significantly different ($p < 0.05$).

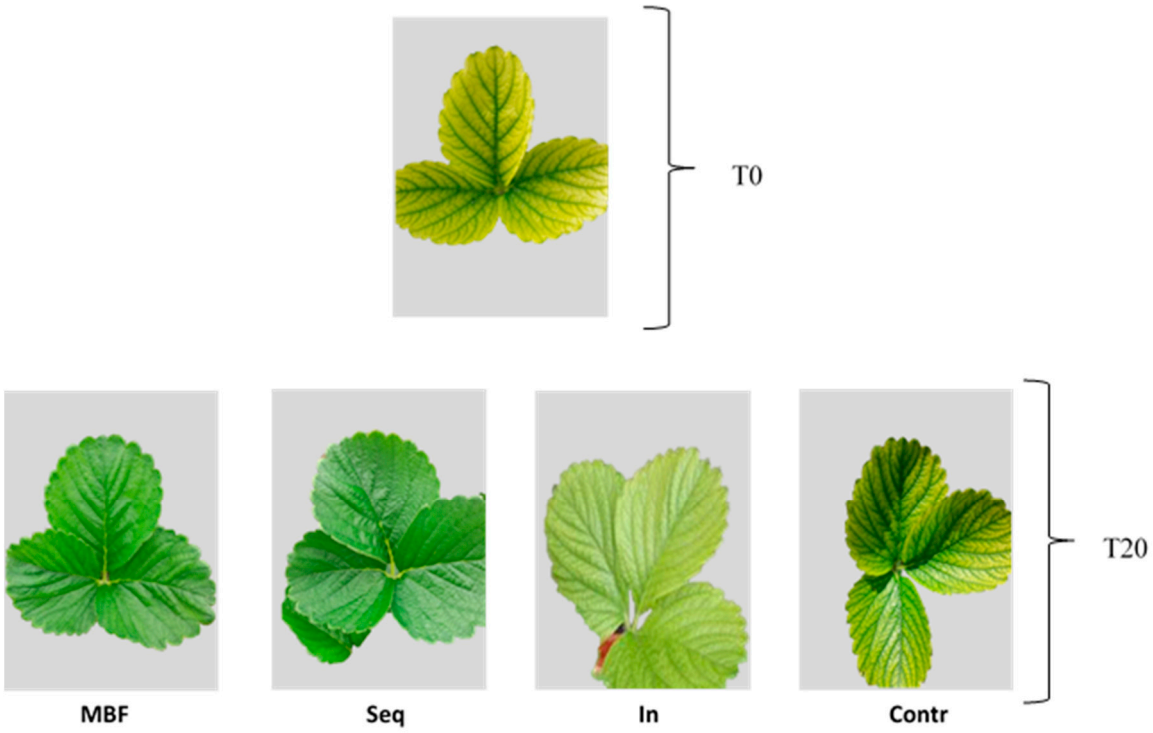


**Figure 5.** Chlorosis symptoms in leaves of strawberry plants at T0 (before treatment) and T20 (20 days after treatment).

The enzymatic results of FC-R activities are shown in Figure 6. The results were consistent with the increased enzymatic activity in the control thesis subjected to chlorosis during the experimental period. In contrast, the roots treated with Seq and MBF showed, already at 5 days after the treatment, enzymatic activity values that were similar among them, and significantly lower with respect to the control and In (Figure 6). However, at 15 and 20 days after the treatments, the In-treated plants showed values significantly lower than those measured in the control, although these activities were always higher than those obtained with Seq and MBF treatments (Figure 6).

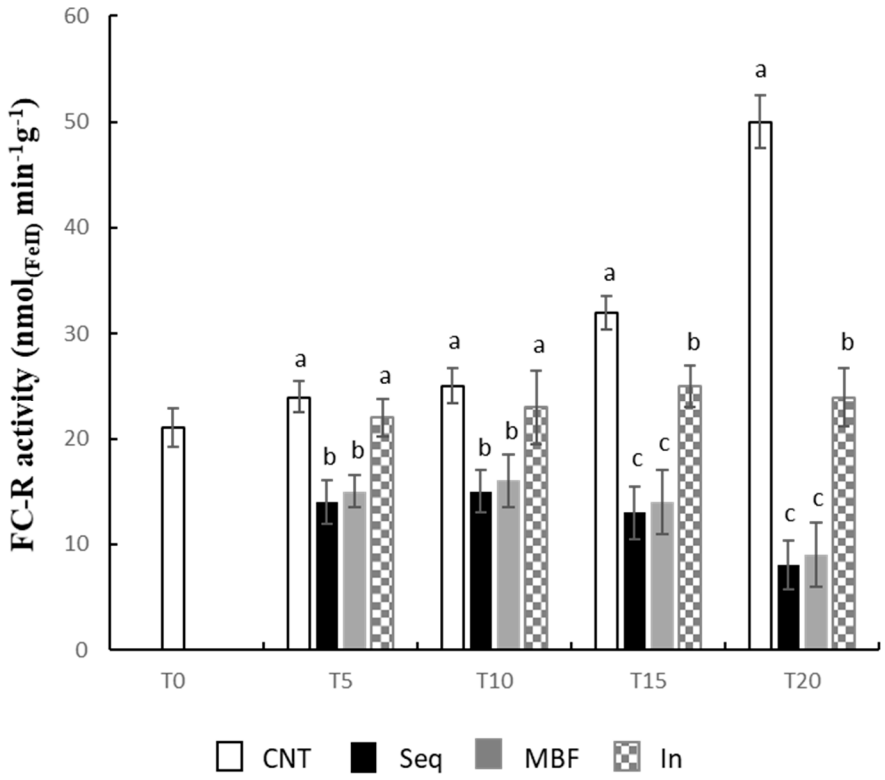


**Figure 6.** Fe chelate reductase activity (FC-R) in roots of strawberry plants. Error bars indicate standard deviation ($n$ = 5). Sampling was performed at T0 (before treatments), T5 (5 days after the treatment), T10 (10 days after the treatment), T15 (15 days after the treatment); T20 (20 days after the treatment). CNT: control, Seq: sequestrene, MBF: Microorganism-based formulation, In: inoculum of MBF. Values within each sampling time followed by different letters are significantly different ($p < 0.05$).

## 4. Discussion

Many authors have focused on the study of the most appropriate strategies to overcome the iron deficiency chlorosis in strawberries [43–47]. In our experimental work, sequestrene efficiently counteracted, within 20 days, the iron deficiency induced in strawberries, in accordance with the evidence that iron chelates are the most widely accepted methods to overcome chlorosis [45,48,49]. The effects of treatments in iron deficiency conditions were evaluated by monitoring several parameters associated to chlorosis, and often involving secondary effects resulting from the complex interactions of Fe with other elements and various soil and environmental factors [50]. These results showed that the treatment with MBF was highly efficient in mitigating the effects of Fe-deficiency induced in strawberry seedlings, reaching results comparable to those obtained using sequestrene at the end of the experimental period by increasing fresh and dry weights (Figures 1 and 2), the absorption of iron (Figure 3), and the chlorophyll content (Figure 4). These data also suggest that a different mechanism of action on the strawberry occurred between MBF and sequestrene. Indeed, already five days after the treatment, sequestrene induced a fast

response when compared to treatment with MBF, resulting in a greater growth, above all at leaf dry weight level (Figure 2A). These results are in accordance with Gilbert [51], who found that when iron deficiency occurs in strawberries, sequestrene can alleviate the negative effects within few days. These prompt effects of sequestrene may be partially attributed to the presence of the commercial formulation of nitrogen and potassium (as detailed in Materials and Methods), which is readily available for the plants. Moreover, the recovery of chlorotic strawberry plants rapidly occurs in response to the availability of iron [38].

The effects on evaluated parameters of strawberry plants treated with MBF were slightly delayed by about five days, which was presumably related to the evidence that in this case the overcoming chlorosis effects was exclusively due to the greater availability of iron generated by the synergy between the inoculated telluric microorganisms, occurring in the commercial formulation, and the presence of available and potentially available iron. On the other hand, in strawberry plants treated only with the inoculum (In), the overcoming effects were slower than the complete commercial formulation (MBF). Therefore, these results suggest that strawberry plants, in this case, were able to use only the native iron of the soil, made available by the phytosiderophores produced by microorganisms present in the inoculum. Although the complete formulation of MBF raised better results, the formulation containing only the microorganisms (In) also acted against chlorosis effects when compared to the control (Figures 1–5). In particular, the mainly evident effects concerning root parameters (Figures 1B, 2B, 3B and 6) were probably due to the mycorrhized roots, which putatively can have a significant benefit in counteracting Fe-deficiency. Therefore, the positive effects against chlorosis observed in strawberry plants treated with MBF and In, above all at root level, might be due to a microorganisms-mediated action of these formulations. This hypothesis is supported by Spinelli et al. [47], who found that the treatment of strawberry plants with a commercial biostimulant seaweed extract (Actiwave®) may counteract the effect of chlorosis, reaching results very similar to sequestrene action, by increasing shoot and root dry matter and positively influencing the root associated microbial community [47].

Although to a different extent, both the alternative treatment methods to sequestrene performed on strawberry plants induced metabolic changes, able to counteract chlorosis within a few days or weeks. Similar results were also obtained by resupplying iron in spinach and sugar beet, leading to an increase in chlorophyll concentration and the rate of photosynthesis within few days [52–54]. Strategy I plants, such as strawberries, respond to Fe-deficiency by increasing their ability to reduce Fe (III) to Fe (II), which can be then taken up by a Fe(II) transporter and absorbed by the root. Consequently, the values of Fe chelate reductase activity (FC-R) increase in the roots [12]. Our results showed that FC-R activity increased in control plants along with the experimental ones (Figure 6), underlying the tendency to respond by using strategy I to address Fe-deficiency due to the worsening of chlorotic conditions in untreated strawberry plants. On the contrary, all the performed treatments reduced the values of FC-R activities along with the experimental ones, in accordance with a reduction of chlorosis symptoms (Figures 5 and 6). These results are supported by Pestana et al. [38], who found smaller FC-R activities in chlorotic strawberry plants treated by foliar spray to supply Fe with respect to the untreated control within few days. The complete formulation of MBF and sequestrene quickly lowered FC-R activity values, coupled with a reduction of chlorosis symptoms (Figure 5). As regards the In treatment, the effectiveness in alleviating chlorosis symptoms was later as well as lower than the complete formulation, however after 15 days, strawberry plants coped with the Fe-deficiency. These results are also supported by Ipek et al. [55], who found that a plant-growth-promoting rhizobacteria (PGPR) treatment was able to increase plant resistance in high calcareous soil conditions.

## 5. Conclusions

In conclusion, microorganism-based formulations may represent a valid alternative to sequestrene in strawberry production. An important role is represented by the mi-

croorganism inoculum. Nonetheless, the use of these microorganism-based formulations must be combined with all the agronomic practices able to minimize the Fe-deficiency and maximize the potential of a crop plant, production, quality, and safety, in order to lower synthetic organic, chelate use and reduce the impacts on the environment.

**Author Contributions:** I.P., S.B. and V.S. conducted the field experiment and determinations; V.T. and A.B. were involved in laboratory analyses; V.S. and I.P. contributed to statistical data processing and interpretation; V.S., A.B. and I.P. conceived and planned the experimental protocol, and performed the research supervision; I.P., S.B. and V.M.S. were involved in bibliographic search; I.P., V.S., V.M.S. and A.B. wrote the draft and final manuscript. All authors have read and agreed to the published version of the manuscript.

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